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Endotoxin-induced changes in phospholipid dynamics of the stomach

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ABSTRACT

Background: The gastric mucosa is protected in part by a hydrophobic layer of phosphatidylcholine (PC) that overlies the mucus gel on the stomach. Endotoxin treatment (i.e., lipopolysaccharide [LPS]) results in an apparent disruption of this layer, as evidenced by a reduction in surface hydrophobicity and an increase in transmural permeability. The current studies compared PC and lyso-PC levels in mucus and gastric mucosa before and after LPS treatment, and examined potential mechanisms for surface phospholipid changes.

Methods: Rats were administered LPS (5 mg//kg, intraperitoneally) and samples were collected after 5 h for analysis of PC and its primary degradant, lyso-PC, in the loosely and firmly adherent mucus layers and the mucosa. The dependence of LPS-induced effects on gastric alkalinization, PC synthetic activity, and intestinal reflux material was assessed.

Results: The gastric contents after LPS, which also contained duodenal reflux material, had greatly increased amounts of PC and lyso-PC. The firmly adherent mucus layer was unchanged. The gastric mucosa after LPS revealed significant reductions of PC levels and no change in lyso-PC content. These phospholipid changes were not caused by alkalinization of the stomach or altered PC synthesis. Prevention of duodenogastric reflux by pylorus ligation blocked the LPS-induced increase in luminal lyso-PC and the reduction in mucosal PC.

Conclusions: LPS appears to induce a release of PC from gastric mucosa into the lumen, along with degradation of PC to lyso-PC, without an effect on PC synthesis. Component(s) of intestinal reflux material appear to be required for these effects. The lowered PC levels in gastric mucosa after LPS may contribute to reduced barrier properties of this tissue.

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1. Introduction

It is well established that the luminal surface of certain regions of the gastrointestinal (GI) mucosa has hydrophobic properties that contribute to the barrier characteristics of this frequently challenged tissue [1–3]. The molecular basis for this hydrophobic surface lining appears to be, in part, attributable to a coating of phospholipid, primarily phosphatidylcholine (PC), which orients on the mucus gel surface to form a water-repellant boundary [4,5]. The PC layer on the gastric mucosa is particularly hydrophobic and helps defend that tissue against back-diffusion of secreted gastric acid. Pathologic states such as peptic ulcer disease associated with *Helicobacter pylori* infection or the ingestion of nonsteroidal

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antiinflammatory drugs appear to be associated with an attenuation in the hydrophobic lining of the upper GI tract and disruption of the PC layer [6–9].

Previously, we reported that endotoxic shock in the rat, which was induced by lipopolysaccharide (LPS) treatment, resulted in a time- and dose-dependent reduction of gastric surface hydrophobicity and a corresponding increase in gastric lumen-to-blood permeability of a test substance (fluorescein isothiocyanate–dextran) [10,11]. We attributed the disruption of the hydrophobic PC layer to LPS-induced duodenogastric reflux of upper intestinal contents, such as secretory phospholipase A_2 ($sPLA_2$) and bile salts, both of which can degrade or solubilize a PC layer. We further showed that the gastric luminal $sPLA_2$ was associated with an increase in luminal lyso-PC content [11], another potent gastric damaging agent. Both $sPLA_2$ and lyso-PC are capable of directly damaging the gastric hydrophobic layer *in vivo* [12]. The gastric contents were also considerably alkalinized (pH $\geq$6) after LPS [13], allowing enzymes within the lumen to be more active. Finally, we showed that pretreatment of rats with orally administered PC could prevent permeability changes after LPS [13].

Our results thus far support the theory that LPS-induced duodenogastric reflux and associated gastroparesis with acid inhibition leads to an alkalinized stomach containing enzymes and detergents that degrade the protective hydrophobic (PC) layer. Further, this condition may lead to transduction of injurious agents into the gastric epithelium, a local inflammatory response, and a potential source of continued systemic inflammation. A better understanding of the pathogenesis of this process is needed in order to counter it.

To test the part of our hypothesis regarding degradation of the PC layer, in the current study we have analyzed the levels of PC found in the gastric mucus and mucosa of rats before and after LPS, so that it can be determined whether our theory about disruption of the PC layer can be substantiated *in vivo*. Also, a possible role for gastric alkalinization in gastric injury was approached by use of the proton pump inhibitor omeprazole. Because LPS has been implicated in causing inhibition of PC synthesis in another system, i.e., lung [14,15], we further investigated the *de novo* synthesis of PC in the stomach and the effect on it of LPS. Finally, we tested by surgical manipulation (pylorus ligation) the importance of duodenogastric reflux of intestinal contents on PC levels altered by LPS.

2. Materials and methods

2.1. Chemicals

LPS (*Escherichia coli* 0111:B4) and phospholipid standards for thin layer chromatography (TLC) were obtained from Sigma Chemical Co (St Louis, MO). Omeprazole was purchased from LKT Laboratories Inc (St Paul, MN). ^{3}H-choline (60–90 Ci/mmol) and ^{3}H-S-adenosylmethionine (80 Ci/mmol) were purchased from Perkin Elmer (Boston, MA).

2.2. Animal studies

All animal studies were approved by the institutional Animal Welfare Committee, which adheres to the National Institutes of Health guidelines for animal care and use. Animals were housed in our public health service- and association for assessment and accreditation of laboratory animal care-approved facility. Sprague-Dawley rats weighing 150–250 g were used (Harlan Sprague-Dawley, Indianapolis, IN). Rats were fasted overnight to ensure an empty stomach. LPS was administered at 5 mg/kg, intraperitoneally. After 5 h, animals were euthanized just prior to collection of fluid and tissue, as previously described [11].

The gastric surface is protected by a variable layer of mucus, composed of loosely and firmly adherent mucus gel [16–18]. The two layers of mucus were collected according to a procedure that was used to identify phospholipid classes and molecular species within the layers [16,19]. Briefly, loosely adherent mucus and fluid gastric contents were collected by flushing the isolated stomach 3–4 times with a total of 20 mL water within a 5-min period. Next, firmly adherent mucus was removed by filling the stomach with 0.5 M saline, placing the stomach in a plastic bag, and keeping it in ice for 20 min, after which the contents were quickly flushed with a total of 20 mL saline. We confirmed in preliminary histologic experiments that this process did not cause disruption of the underlying mucosa. Lastly, the stomach was opened and the mucosal surface was scraped off with the edge of a glass slide for collection of gastric mucosal tissue.

The effect of gastric alkalinization was investigated by treating rats with saline or omeprazole (75 mg/kg, twice daily for 4 d). Tissue collection from fasted rats was as described above.

2.3. PC analysis

The fluid and mucus collections were mixed with 4× volume of chloroform:methanol (2:1) and extracted for total lipid by the Folch procedure [20]. The mucosal tissue was extracted for total lipid after homogenization in chloroform:methanol (2:1). Lipid extracts were dried under nitrogen gas and resuspended in chloroform for spotting onto TLC plates. Elution of plates for phospholipids (PC and lyso-PC) and analysis of phosphorus content was as previously described [11]. Values for PC and lyso-PC in the mucus layers were calculated as micrograms of phospholipid per total stomach volume, whereas those values in the gastric mucosal tissue were expressed as micrograms of phospholipid per gram of collected mucosa.

2.4. PC synthesis

Gastric mucosal tissue was homogenized in buffer (20 mM Tris, 100 mM sodium chloride, 2 mM EDTA, and 10 μL/mL protease inhibitor cocktail (Sigma P8340, pH 7.4)) to 250 mg/mL. The homogenate was centrifuged in the cold at 1000*g* for 5 min. The supernatant was used in subsequent analyses.

For determination of PC synthesis through the CDP-choline pathway [21], homogenate (50 μL) was mixed with 0.1 mL ^{3}H-choline (20 μCi/mL) in 1 mM choline to a total volume of 1 mL. Samples were incubated at 37°C for 60 min. The reaction was stopped by addition of 4 mL chloroform:methanol (2:1) and subsequent lipid extraction and TLC as described above. Spots containing PC were scraped into scintillation vials with 10 mL counting fluid and counted for radioactivity. Values were

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